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TWENTY FOUR HOURS RUMEN FERMENTATION PATTERNS ON DIETS DIFFERING IN CARBOHYDRATE COMPOSITION

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ABSTRACT

In the present experiment, two fattening rations differing in polysaccharide composition were compared with 12 double muscled young bulls of the Belgian Blue Breed weighing about 420 kg. The first group (6 bulls) received a diet containing a high proportion of pectine provided by 0.50 sugar beet pulp (P) while the second diet was rich in starch (0.50 barley - B). Rumen fluid was sampled every 2 hours over a 24 h period and analyzed for pH, volatile fatty acids (VFA), ammonia N, alpha-amino-N and glucose. In addition to rumen sampling 24 h plasma profiles of urea N, glucose, alpha-amino-N and insulin and N-balance were measured in the preceeding and following weeks. The initial pH (6.9) decreased after each meal for both groups resulting in minima of 5.4 and 5.6 for B and P respectively and increased during the night. For total VFA concentrations, higher maxima were observed for the B group : respectively 144 and 152 mmoles/l after the first meal and 160 and 190 after the second meal. The P group featured a preponderating acetic acid fermentation while for the B group fermentation was mainly propionic. Rumen ammonia N decreased after each meal (135 to 50 mg/l for the P group and to as low as 13 mg/l for the B group after the first meal) and increased again to about 130 mg/l during the night. Alpha-amino-N rose after feeding and during the night period high values were obtained (100 and 130 mgN/l for P and B groups respectively). Glucose followed a similar pattern, the highest values being obtained with the B group. Generally, metabolite patterns indicated slower kinetics with the P than with the B group. At the venous blood level glucose and urea were higher with the P group while alpha-amino-N and insulin were somewhat higher with the B group. In conclusion, slower and apparently "better tuned" rumen fermentations, higher microbial growth (as measured by urinary allantoin excretion) as well as higher N-balance agree with the higher growth rates obtained with the sugar beet pulp diet.

INTRODUCTION

The rumen is a highly complex and integrated fermentation system where bacteria and protozoa grow and proliferate, providing the host animal with useful energetic fermentation end-products such as volatile fatty acids

(VFA) and high quality microbial protein. In this respect the microbes rely on the host animal's dietary intake to satisfy their requirements for energy (essentially carbohydrates) and nitrogen (dietary protein or non protein N). Consequently, rumen content is a mixture of microbes and ingested and partially degraded substrate material, mainly dietary polysacharides and protein, intermediate products of fermentative degradation, such as polypeptides, amino acids and sugars (mainly glucose) and end-products such as volatile fatty acids (acetic, propionic and butyric acids) and ammonia, which are either absorbed through the rumen epithelium and used by the animal (VFA) or serve as precursors for microbial growth (amino acids, ammonia). The functioning of the rumen as a fermentation vessel is a complex array of several consecutive and simultaneous processes. Substrate (feed) is mixed with saliva and arrives in the ruminal pouch where extensive mixing takes place by contraction of the "vessel walls". Feed boluses are regurgitated and go back and forth several times between the rumen and the animal's mouth (i.e. the rumination process). Undigested and partially digested particles move out to the subsequent parts of the digestive tract at variable speeds and times. Fermentation products are either absorbed irreversibly or absorbed and partially recycled back into the rumen after hepatic transformation (ammonia as urea). All these processes are under neuro-endocrine control so the "fermentation vessel" is an integrated part of the whole animal and rumen metabolism is influenced by and influences intermediary metabolism.

This work is part of a study on the effects of nutritional factors on endocrine parameters in relation to beef meat production. As rumen fermentation patterns are known to be greatly influenced by the carbohydrate composition of the diet the effects of varying this composition on rumen fermentations and endocrine and metabolic status were studied. The present communication deals mainly with the observations on rumen fermentations.

MATERIAL AND METHODS

Animals : 12 double muscled young bulls of the Belgian Blue Breed were alotted to 2 groups of 6 animals. All animals were fistulated with a dorsal rumen canula. Average weights at the rumen sampling day were 422.3 and 420.8 kg respectively.

Diets : two fattening diets differing in carbohydrate composition were given to the 2 groups. Both diets were composed of soya bean meal, barley, dried sugar beet pulp, hay and a mineral supplement. The first group received a diet containing a high proportion of pectin, provided by 0.50 sugar beet pulp (P) while the second diet was rich in starch (0.50 barley - B). The composition of the two diets is given in table 1. The experimental diets were given in 2 equal meals at 8 am and 16 pm, directly after the corresponding rumen sampling.

Measurements : after a 10 weeks stabilisation on the experimental diets rumen fluid was sampled every 2 h during a whole 24 h period. Sampling started at 6 am and was finished at 6 am of the next day. Rumen liquid was analyzed for pH, volatile fatty acids (acetic, propionic, butyric, n and isovaleric acids), ammonia, alpha-amino-N and glucose. At 8 am and 12 am (4 h after the morning meal) separate collection of rumen content (solid + liquid phases) was performed in order to determine partitioning of dry matter and N in the two phases. One and half week before rumen sampling N-balance was measured over a 10 days measurement period. N-retention and urinary allantoin excretion were estimated. One week after rumen sampling

venous blood samples were taken every 20 minutes over a 24 h period and plasma concentrations of urea, glucose, alpha-amino-N, growth hormone and insulin measured.

Chemical analysis : pH was measured by potentiometry, VFA were determined by gas chromatography on a butanediolsuccinate column, ammonia, alpha-amino-N, glucose, urea by Autoanalyzer methods using respectively the Berthelot reaction, the TNBS reaction, the o-toluidine method and the diacetylmonoxime reaction. Nitrogen and protein by Kjeldahl digestion on a Tecator Block and subsequent colorimetry using the same reaction as for ammonia. Allantoin was determined by an automated version of the method of Borchers (1977). Plasma GH and insulin were determined by RIA.

Table 1. - COMPOSITION OF THE TWO EXPERIMENTAL DIETS

	Dried sugar beet pulp	Barley	Soya bean meal	Hay	Mineral mixture
	% of total		kg/day		
Group P	45-55	20	1,5	1	0,14
Group B	20	45-55	1,5	1	0,14

The quantities of respectively dried sugar beet pulp (group P) and barley (group B) were varied in function of animal weight in order to achieve ad libitum feeding.

RESULTS

Evolution patterns of pH, total VFA concentrations, mean VFA molar proportions, ammonia N, alpha-amino-N and glucose in rumen fluid during the 24 h period are depicted in figures 1 to 6.

pH :

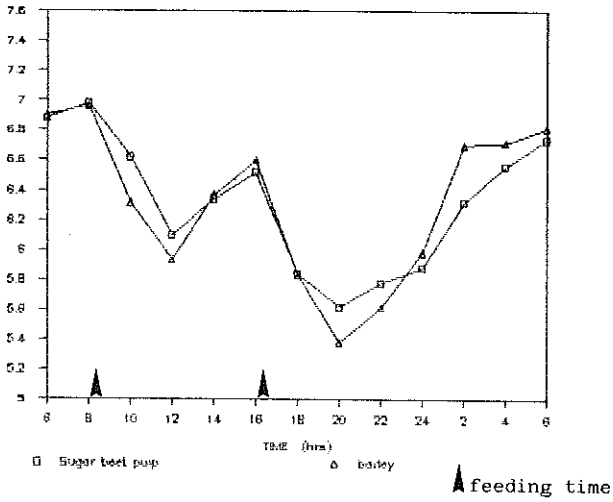

Fig. 1.- EVOLUTION PATTERN OF pH.

pH decreased immediately after each meal, reached a minimum after 4 h and increased again afterwards. The second minimum was lower than the first : 5,6 vs 6,1 and 5,4 vs 5,9 respectively for P and B groups. Such pattern indicates faster, more "explosive" fermentations for the B diet compared to slower and more sustained fermentations for the P group.

Concentrations of end-product :

- Volatile fatty acids

Total VFA concentrations followed a somewhat mirror image pattern with pH, the maxima occurring at the minima for pH. Correspondingly highest concentrations were observed 4 h after the second meal : 160 vs 144 and 189 vs 152 mmol/l respectively for P and B. Fall and rise curves were also steeper for the latter.

Molar proportions of VFA revealed a predominating acetic acid fermentation for the pulp diet while the B diet produced more propionic acid.

- Ammonia

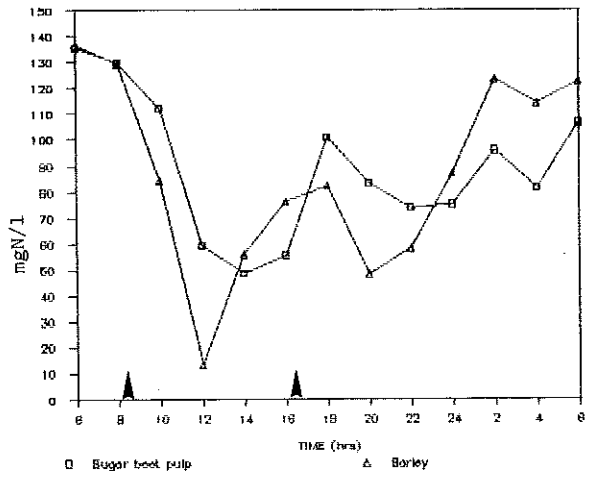

Fig. 2.- EVOLUTION PATTERN OF NH_3

Ammonia N concentration was high before the morning meal (135 mgN/l) in both treatments, and decreased afterwards, the steepest descent again being observed with the barley diet. Minima coincided with pH minima and VFA maxima at 4 h after feeding for the B group (14 mgN/l). For the P group ammonia was low after 4 h (50 mgN/l) but the actual minimum (40 mgN/l) was reached only after 6 h. After the second meal ammonia concentrations still continued to increase and fell only after 2 h to minima at respectively 4

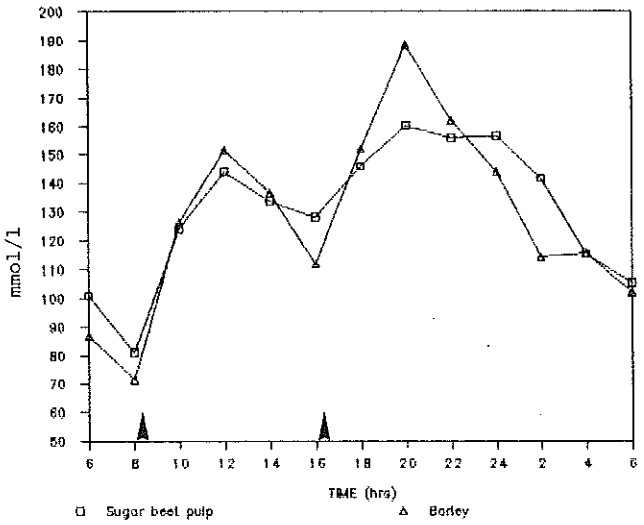

Fig. 3.- EVOLUTION PATTERN OF TOTAL VFA CONCENTRATIONS.

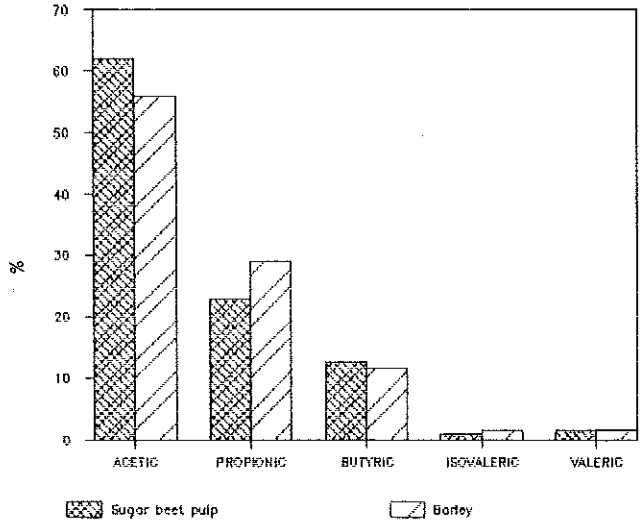

Fig. 4.- MEAN MOLAR PROPORTIONS OF VFA.

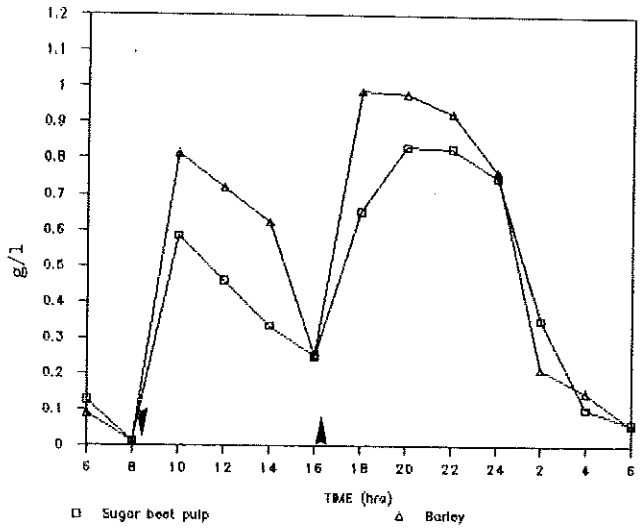

Fig. 5.- EVOLUTION PATTERN OF GLUCOSE.

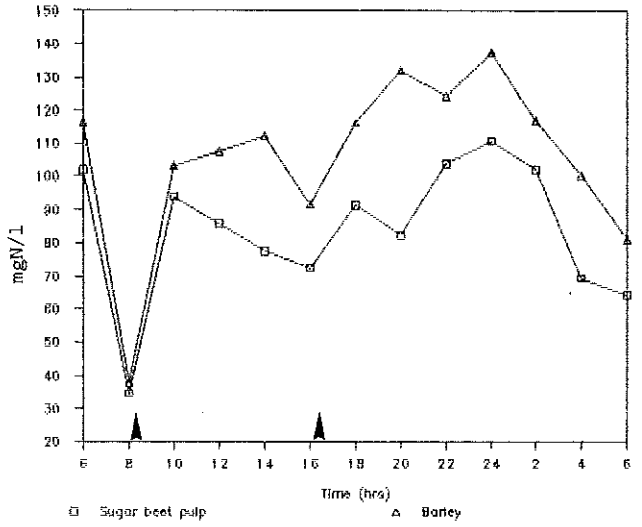

Fig. 6.- EVOLUTION PATTERN OF ALPHA-AMINO-N.

and 6 h after the meal for the B and P diets. The second minima were higher than the first ones and persisted longer (about 75 mgN/l for P and 50 mgN/l for B). During the night period ammonia concentrations rose to high levels. The P pattern seemed to be shifted back in time with regard to B indicating again slower and more extended fermentations.

Concentrations of intermediate products : from respectively polysaccharide and protein degradation.

Glucose : glucose concentrations were low (about 100 mg/l) before the first meal and during the last part of the night (from 2 am), and increased sharply after each meal, the maxima being higher in the barley group. The highest concentrations were observed after the second meal and till midnight : 700 to 900 mg/l.

Alpha-amino-N : alpha-amino-N concentrations displayed also maxima after the meals although the patterns were less pronounced than for glucose. Concentrations were high at night and decreased from 24 pm to 8 am (see decrease between the first two samplings). After the first meal alpha-amino-N increased sharply for both treatment groups. From 2 h after the first meal to the morning period next day alpha-amino-N remained higher for the barley group displaying mean differences of 20 to 40 mgN/l.

DISCUSSION

The concentration of any metabolite in rumen liquid is the net result of the balance between positive and negative factors such as the rates of formation and disappearance, dilution and recycling. The formation rate depends on the velocities of the degradation reactions of both dietary and microbial substrates, the solubility and availability of precursor material and the activities of several enzymes. Metabolites disappear from the rumen by absorption through rumen epithelium, utilisation as precursor in synthetic reactions of microbes, and transport to subsequent parts of the digestive tractus. Taking into account these constraints with regard to a quantitative interpretation, the present communication could nevertheless represent an integrated approach of rumen fermentation patterns of several metabolites measured simultaneously over a complete diurnal 24 h period, with young bulls, on meat production diets and in relation to parameters of intermediary metabolism.

For most metabolites the overall patterns were different between the two diets : with the barley group maxima were higher and the shape of the curve sharper (e.g. VFA, glucose, alpha-amino-N) with deeper minima (e.g. pH, ammonia), while for the pulp group patterns were somewhat flatter. This could be related to a faster fermentability of the carbohydrate fraction (e.g. higher glucose concentrations in the rumen) and nitrogen fraction (higher soluble N content) of the barley diet. The latter difference should be accounted for exclusively to the composition of the basal feeds (pulp vs barley) since total crude protein was equal for both rations (about 15%) as was the quantity of soya bean meal in the two diets (1,5 kg/d). Apparently the higher solubility of barley protein results in higher concentrations of amino acids and small peptides which were measured as alpha-amino-N by the TNBS method. On the other hand, deamination with ammonia production should be slower with the barley diet, as ammonia concentration, apart from the night period (24 pm to 6 am) was lower. Another explanation could be a faster incorporation of ammonia into microbial protein. However, this contrasts with the results obtained for microbial N production as estimated

by urinary allantoin excretion. Under the assumptions that nucleic acid N represents 19% of microbial N, that 80% of nucleic acid N is absorbed and that 30% of nucleic acids are converted to allantoin (Bickel et al., 1986), 1 gN allantoin can be converted to 21,9 g microbial N. In this experiment allantoin excretions were 5 g and 3,8 g/d for the P and B groups respectively. This accounts for a microbial N synthesis of respectively 38 and 29 g/d. Expressed on a metabolic weight base this amounts to respectively 52,3 and 40,3 mg/kgLW$^{0.75}$ /d, which compares to the 72 mg in steers and 26 mg in sheep found by Fujihara et al. (1987) with the same method.

Ammonia concentrations were generally low with both rations. Highest values were obtained during the night, and generally, when a sufficiently long period is elapsed after the meal e.g. when apparently energy supply was limiting to support a high incorporation rate of ammonia into microbial protein and increased lysis of microbes occurred (Van Nevel and Demeyer, 1983). Dehareng et al. (1987) observed similar high ammonia concentrations 7 to 8 h after the last meal in a fasted dry cow. Ammonia patterns were different after the two meals. After the first meal ammonia concentrations fell immediately while after the second meal a temporary increase was followed by a decrease. Apparently ammonia utilisation started immediately after the morning meal when rumen liquid was most "potent" and concentrated and the supply of energetic feed immediately turned on microbial synthesis. The higher supply of readily available carbohydrates in the barley diet explains the steeper fall of ammonia concentrations. During the day, especially with the B group ammonia even fell below 50 mg/l, reported by Satter and Slyter (1974) as the limiting ammonia concentration for microbial growth. On the other hand too high ammonia concentration are a burden to the host animal forcing it to an increased urea synthesis in the liver. Significant correlation ($P < 0,01$) were found between rumen ammonia and blood urea with a time delay of 3 h. The best correlation is obtained for the barley diet. Finally one could wonder if the "best" fermentation pattern is not the "flatter" one, as observed for the pulp diet. This could be an indication of an optimal "tuning" between degradative processes, producing precursor metabolites, and synthesis reactions, which use these molecules.

VFA concentrations showed higher maxima for the barley diet although overall daily concentrations were similar (130,2 mmol/l for P and 127,9 for B). There was however a difference in fermentation type : the P diet produced a more acetic fermentation while for the B group more propionic acid was formed. This could be related to substrate type (starch vs pectin) but also to possible differences in dilution rates caused by a difference in texture and volume of the rumen content. Higher dilution rates have been shown to be correlated with a higher proportion of propionic acid (Van Nevel and Demeyer, 1983). On the other hand the higher content in acetic acid observed with the P diet correlates with higher microbial N yields as suggested by Harrison et al. (1975) and Thomson et al. (1978).

VFA concentrations were also related to the hormonal status of the animal. Blood insulin concentrations were highest with the barley group and were correlated significantly ($P < 0,01$) with the propionic acid concentration in the rumen. As propionic acid is the major glucogenic precursor in ruminants this could be expected. However, insulin was poorly related to blood glucose (r non significant), suggesting a more direct triggering of insulin response by propionic acid.

In conclusion, rumen fermentations are influenced by the carbohydrate composition of the diet and influence conversely the host animal's intermediate metabolism and hormonal status.

ACKNOWLEDGEMENTS

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RESULTS

Evolution patterns of pH, total VFA concentrations, mean VFA molar proportions, ammonia N, alpha-amino-N and glucose in rumen fluid during the 24 h period are depicted in figures 1 to 6.

pH :

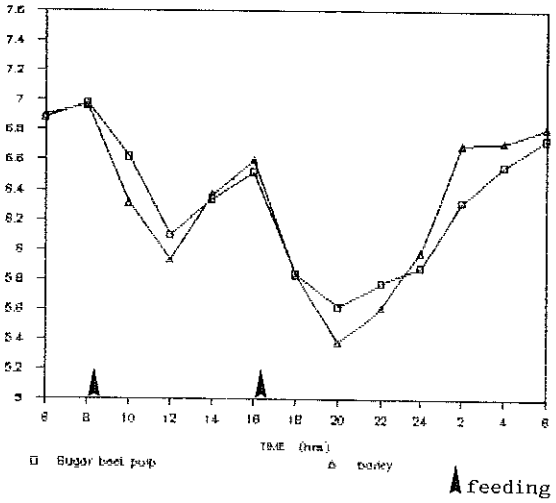

Fig. 1.- EVOLUTION PATTERN OF pH.

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Concentrations of end-product :

- Volatile fatty acids

Total VFA concentrations followed a somewhat mirror image pattern with pH, the maxima occurring at the minima for pH. Correspondingly highest concentrations were observed 4 h after the second meal : 160 vs 144 and 189 vs 152 mmol/l respectively for P and B. Fall and rise curves were also steeper for the latter.

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- Ammonia

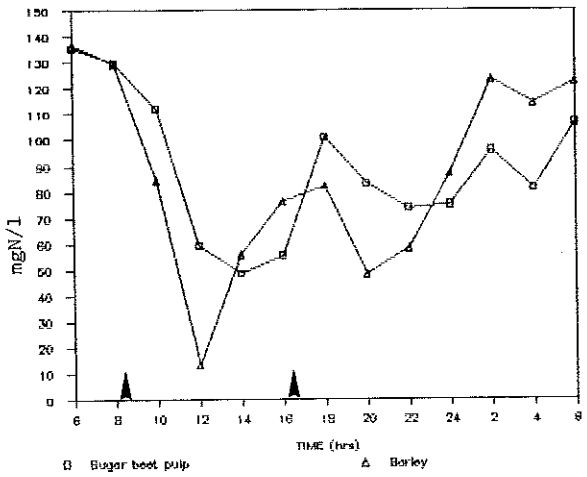

Fig. 2.- EVOLUTION PATTERN OF NH_3

Ammonia N concentration was high before the morning meal (135 mgN/l) in both treatments, and decreased afterwards, the steepest descent again being observed with the barley diet. Minima coincided with pH minima and VFA maxima at 4 h after feeding for the B group (14 mgN/l). For the P group ammonia was low after 4 h (50 mgN/l) but the actual minimum (40 mgN/l) was reached only after 6 h. After the second meal ammonia concentrations still continued to increase and fell only after 2 h to minima at respectively 4

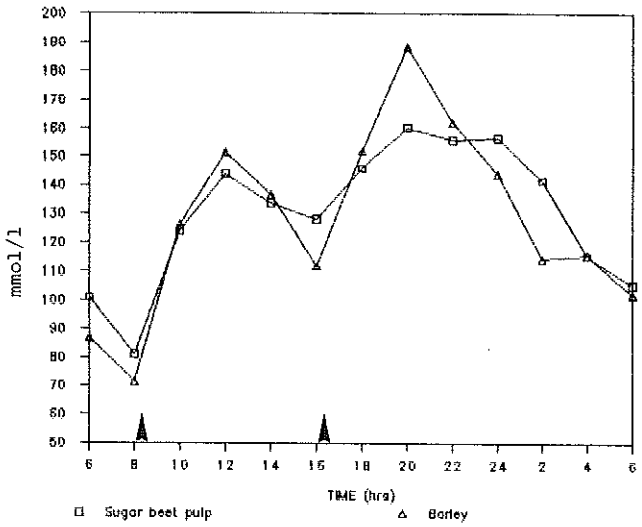

Fig. 3.- EVOLUTION PATTERN OF TOTAL VFA CONCENTRATIONS.

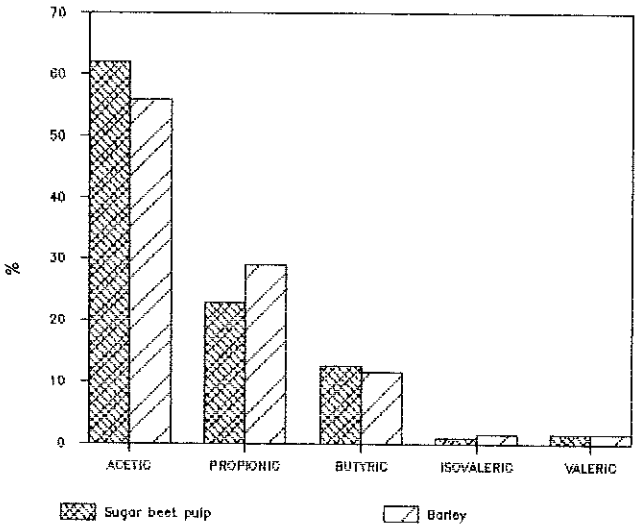

Fig. 4.- MEAN MOLAR PROPORTIONS OF VFA.

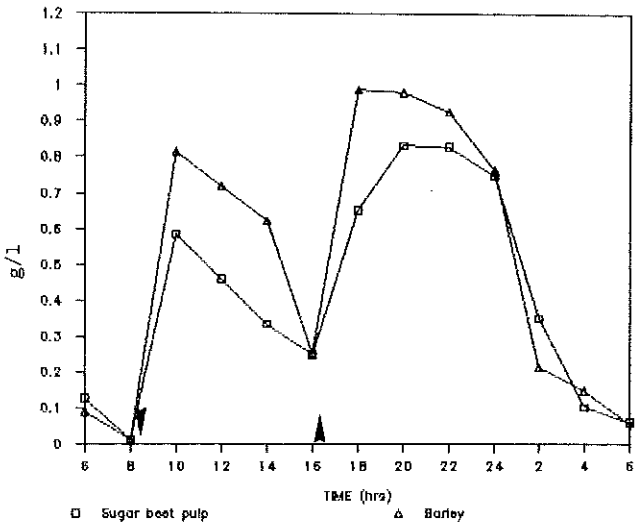

Fig. 5.- EVOLUTION PATTERN OF GLUCOSE.

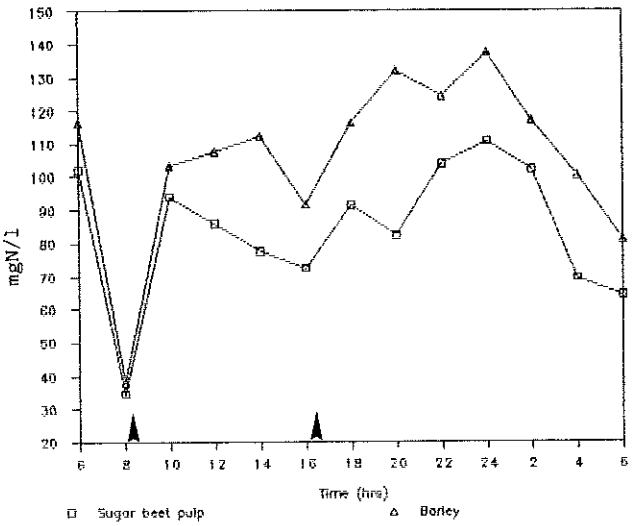

Fig. 6.- EVOLUTION PATTERN OF ALPHA-AMINO-N.

and 6 h after the meal for the B and P diets. The second minima were higher than the first ones and persisted longer (about 75 mgN/l for P and 50 mgN/l for B). During the night period ammonia concentrations rose to high levels. The P pattern seemed to be shifted back in time with regard to B indicating again slower and more extended fermentations.

Concentrations of intermediate products : from respectively polysaccharide and protein degradation.

Glucose : glucose concentrations were low (about 100 mg/l) before the first meal and during the last part of the night (from 2 am), and increased sharply after each meal, the maxima being higher in the barley group. The highest concentrations were observed after the second meal and till midnight : 700 to 900 mg/l.

Alpha-amino-N : alpha-amino-N concentrations displayed also maxima after the meals although the patterns were less pronounced than for glucose. Concentrations were high at night and decreased from 24 pm to 8 am (see decrease between the first two samplings). After the first meal alpha-amino-N increased sharply for both treatment groups. From 2 h after the first meal to the morning period next day alpha-amino-N remained higher for the barley group displaying mean differences of 20 to 40 mgN/l.

DISCUSSION

The concentration of any metabolite in rumen liquid is the net result of the balance between positive and negative factors such as the rates of formation and disappearance, dilution and recycling. The formation rate depends on the velocities of the degradation reactions of both dietary and microbial substrates, the solubility and availability of precursor material and the activities of several enzymes. Metabolites disappear from the rumen by absorption through rumen epithelium, utilisation as precursor in synthetic reactions of microbes, and transport to subsequent parts of the digestive tractus. Taking into account these constraints with regard to a quantitative interpretation, the present communication could nevertheless represent an integrated approach of rumen fermentation patterns of several metabolites measured simultaneously over a complete diurnal 24 h period, with young bulls, on meat production diets and in relation to parameters of intermediary metabolism.

For most metabolites the overall patterns were different between the two diets : with the barley group maxima were higher and the shape of the curve sharper (e.g. VFA, glucose, alpha-amino-N) with deeper minima (e.g. pH, ammonia), while for the pulp group patterns were somewhat flatter. This could be related to a faster fermentability of the carbohydrate fraction (e.g. higher glucose concentrations in the rumen) and nitrogen fraction (higher soluble N content) of the barley diet. The latter difference should be accounted for exclusively to the composition of the basal feeds (pulp vs barley) since total crude protein was equal for both rations (about 15%) as was the quantity of soya bean meal in the two diets (1,5 kg/d). Apparently the higher solubility of barley protein results in higher concentrations of amino acids and small peptides which were measured as alpha-amino-N by the TNBS method. On the other hand, deamination with ammonia production should be slower with the barley diet, as ammonia concentration, apart from the night period (24 pm to 6 am) was lower. Another explanation could be a faster incorporation of ammonia into microbial protein. However, this contrasts with the results obtained for microbial N production as estimated

by urinary allantoin excretion. Under the assumptions that nucleic acid N represents 19% of microbial N, that 80% of nucleic acid N is absorbed and that 30% of nucleic acids are converted to allantoin (Bickel et al., 1986), 1 gN allantoin can be converted to 21,9 g microbial N. In this experiment allantoin excretions were 5 g and 3,8 g/d for the P and B groups respectively. This accounts for a microbial N synthesis of respectively 38 and 29 g/d. Expressed on a metabolic weight base this amounts to respectively 52,3 and 40,3 mg/kgLW$^{0.75}$/d, which compares to the 72 mg in steers and 26 mg in sheep found by Fujihara et al. (1987) with the same method.

Ammonia concentrations were generally low with both rations. Highest values were obtained during the night, and generally, when a sufficiently long period is elapsed after the meal e.g. when apparently energy supply was limiting to support a high incorporation rate of ammonia into microbial protein and increased lysis of microbes occurred (Van Nevel and Demeyer, 1983). Dehareng et al. (1987) observed similar high ammonia concentrations 7 to 8 h after the last meal in a fasted dry cow. Ammonia patterns were different after the two meals. After the first meal ammonia concentrations fell immediately while after the second meal a temporary increase was followed by a decrease. Apparently ammonia utilisation started immediately after the morning meal when rumen liquid was most "potent" and concentrated and the supply of energetic feed immediately turned on microbial synthesis. The higher supply of readily available carbohydrates in the barley diet explains the steeper fall of ammonia concentrations. During the day, especially with the B group ammonia even fell below 50 mg/l, reported by Satter and Slyter (1974) as the limiting ammonia concentration for microbial growth. On the other hand too high ammonia concentration are a burden to the host animal forcing it to an increased urea synthesis in the liver. Significant correlation ($P < 0,01$) were found between rumen ammonia and blood urea with a time delay of 3 h. The best correlation is obtained for the barley diet. Finally one could wonder if the "best" fermentation pattern is not the "flatter" one, as observed for the pulp diet. This could be an indication of an optimal "tuning" between degradative processes, producing precursor metabolites, and synthesis reactions, which use these molecules.

VFA concentrations showed higher maxima for the barley diet although overall daily concentrations were similar (130,2 mmol/l for P and 127,9 for B). There was however a difference in fermentation type : the P diet produced a more acetic fermentation while for the B group more propionic acid was formed. This could be related to substrate type (starch vs pectin) but also to possible differences in dilution rates caused by a difference in texture and volume of the rumen content. Higher dilution rates have been shown to be correlated with a higher proportion of propionic acid (Van Nevel and Demeyer, 1983). On the other hand the higher content in acetic acid observed with the P diet correlates with higher microbial N yields as suggested by Harrison et al. (1975) and Thomson et al. (1978).

VFA concentrations were also related to the hormonal status of the animal. Blood insulin concentrations were highest with the barley group and were correlated significantly ($P < 0,01$) with the propionic acid concentration in the rumen. As propionic acid is the major glucogenic precursor in ruminants this could be expected. However, insulin was poorly related to blood glucose (r non significant), suggesting a more direct triggering of insulin response by propionic acid.

In conclusion, rumen fermentations are influenced by the carbohydrate composition of the diet and influence conversely the host animal's intermediate metabolism and hormonal status.

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